PATENT COOPERATION TREATY

10 Rec'd PCT/PTO 28 JUN 2004

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

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PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

Date of mailing *(day/month/year)*	16.04.2004
Applicant's or agent's file reference	**IMPORTANT NOTIFICATION**
POP-0009.PCT	

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/IB 02/05581	23.12.2002	29.12.2001

Applicant
POLYPEPTIDE LABORATORIES A/S, et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

The applicant's attention is drawn to Article 33(5), which provides that the criteria of novelty, inventive step and industrial applicability described in Article 33(2) to (4) merely serve the purposes of international preliminary examination and that "any Contracting State may apply additional or different criteria for the purposes of deciding whether, in that State, the claimed inventions is patentable or not" (see also Article 27(5)). Such additional criteria may relate, for example, to exemptions from patentability, requirements for enabling disclosure, clarity and support for the claims.

Name and mailing address of the international preliminary examining authority:	Authorized Officer
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PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
POP-0009.PCT		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/IB 02/05581	23.12.2002	29.12.2001

International Patent Classification (IPC) or both national classification and IPC

C07K1/30, C07K1/30

Applicant

POLYPEPTIDE LABORATORIES A/S, et al.

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 9 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
07.07.2003	16.04.2004

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/IB 02/05581

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1, 2, 2a, 3-7 filed with telefax on 12.03.2004

Claims, Numbers

1-10 filed with telefax on 12.03.2004

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/IB 02/05581

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-10
	No:	Claims	-
Inventive step (IS)	Yes:	Claims	1-9
	No:	Claims	10
Industrial applicability (IA)	Yes:	Claims	1-10
	No:	Claims	-

2. Citations and explanations

see separate sheet

INTERNATIONAL PRELIMINARY EXAMINATION REPORT - SEPARATE SHEET

International application No. PCT/IB 02/05581

Re Item I

Basis of the report

A.1 The amendments (see below) do not introduce subject-matter which extends beyond the content of the application as originally filed.

A.2 The unclear term "ethyl isopropionate" has been deleted from the list of alternative polar compounds for the definition of the precipitation solvent mixture in claim 1. The description has been amended accordingly (see page 3, line 8).

A.3 The original claim 6 has been deleted and original claims 7-11 have been renumbered in new claims 6-10.

A.3 The relevant prior art has been acknowledged in the description (on page 2a).

A.4 Few typing errors have been corrected.

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. PRIOR ART.

Reference is made to the following documents cited in the International Search Report:

D1: WO 0018423 A;
D2: WO 0055190 A;
D3: EP 0136728 A;
D4: EP 0955308 A;
D5: WO 9110677 A.

1.1 D1 discloses LH-RH peptide analogues and their pharmaceutically acceptable salts for the manufacture of medicaments (see abstract and page 11, line 2). Abarelix is mentioned among the representative peptide analogues (see page 11,

lines 6-9). In particular, D1 discloses the acetate salts of these peptide analogues (see page 11, lines 10-15).

1.2 D2 discloses LH-RH antagonists for the manufacture of medicaments against hormone-dependent tumours and hormone-influenced diseases (see abstract). In specific embodiments, the antagonists are decapeptides and are purified by means of preparative HPLC and freeze-drying (see examples 1-6).

1.3 D3 discloses a process for dissolving peptides and proteins in mixed non-aqueous/aqueous solvents in the presence of crown-ethers (see claim 1). For example, such a process is useful for purification and/or separation purposes (see claim 11). In a specific embodiment, the non-aqueous solvent is ethanol or methanol and the solvent mixture may further comprise ethyl acetate (see claim 5 and page 2, lines 17-25).

1.4 D4 discloses a process for the purification of oligopeptides used for the manufacture of medicaments (see abstract). In particular, the oligopeptides are LH-RH antagonists of 10 amino acids (see page 4, lines 33-45). The purification process involves the hydrochloride salts of the oligopeptides, which are transformed in the corresponding acetate by means of a single-step liquid chromatography (see claim 1). In a preferred embodiment, the hydrochloride salts are obtained by means of lyophilisation (see claim 3).

1.5 D5 discloses a process for the separation and the purification of biomolecules by means of an aqueous/organic solvent system, which undergoes phase separation upon the addition of salts (see claim 1). In preferred embodiments, the biomolecules are proteins (see examples 1-4).

2. NOVELTY (Art. 33(2) PCT) and INVENTIVE STEP (Art. 33(3) PCT).

2.1 The subject-matter of claims 1-9 appears to be novel and to involve an inventive step because the available prior art does not disclose or suggest any process for the purification of peptides from residual organic solvents as claimed.

2.1[a] The claimed purification process mainly differs from the known processes in the use of the precipitation solvent mixture, which comprises both polar and non-polar solvents. In particular, the purification methods disclosed in D3 and D5 applies to enzyme and recombinant proteins, rather than oligopeptides as the claimed

method, and do not involve any precipitation solvent mixture as indicated above, but dialysis and phase partitioning are used for recovering the solubilized proteins (see point 1.3 and 1.5 above). D2 and D4, which concern oligopeptides, disclose chromatographic purification methods with the final lyophilisation of the eluates, but no precipitation step (see points 1.2 and 1.4 above).

2.1[b] Taking D2 or D4 as the relevant state of the art, the problem to be solved can be regarded as the provision of further processes for the purification of oligopeptides from residual organic solvents (see also the paragraph joining pages 1 and 2 of the application).

2.1[c] The claimed process is to be considered as involving an inventive step because the use of a precipitation solvent mixture comprising polar and non-polar solvents has not been suggested in the available prior art, and in particular no dissolution/precipitation procedure has been suggested for solving the problem posed with respect to the residual organic solvents. Even if the dialysis of D3 can be considered a precipitation step (it leads to the precipitation of the protein), it does not involve any non-polar solvent, and D3 does not concern any organic solvent contaminant.

2.2 The subject-matter of claim 10 is novel over the available prior art, because none of the cited documents discloses the monoacetate of the abarelix peptide referred to in the claim.

2.2[a] D1 is considered to represent the relevant state of the art because it discloses pharmaceutically acceptable salts of the abarelix peptide, from which the subject-matter of claim 10 differs in the monoacetate salt form (see point 1.1 above).

2.2[b] In the light of this prior art, the problem to be solved can be regarded as the provision of further pharmaceutically acceptable salts of the abarelix peptide.

2.2[c] The subject-matter of claim 10 cannot be considered as involving any inventive step because D1 and D4 indicate the acetate moiety as a suitable and preferred counter-ion for the salts of this peptide or peptides of the same family and structure (see points 1.1 and 1.4 above).

2.2[d] The monoacetate feature is implicit for the abarelix acetate salt because this peptide presents a single basic group (on the Lys(^{i}Pr) residue). Under pharmaceutically acceptable conditions, this basic group carries a positive charge, which is counter-balanced by an anion in the salts of the peptide. Hence, a single acetate moiety is to be indicated in the chemical formula of the abarelix acetate salt suggested by the prior art, i.e. this salt is inherently a monoacetate.

2.2[e] Provided that two or more pharmaceutically acceptable acetate salt forms exist for the abarelix peptide, the specific choice of the monoacetate form is to be

considered a selection between/among the equivalent alternatives of these acetate salt forms suggested in the prior art. Such a selection can only be regarded as inventive, if the abarelix monoacetate presents unexpected effects or properties (i.e. pharmacological or pharmaceutical properties) with respect to other acetate salt forms of the abarelix peptide. However, no such effects or properties are indicated in the application, which only provides experimental data about the purification process and no evidence of other acetate salt forms. Hence, no inventive step is present in the subject-matter of claim 10.

3. INDUSTRIAL APPLICABILITY (Art. 33(4) PCT).

3.1 Claims 1-9 and 10 relates to methods for the purification of oligopeptides and to an oligopeptide derivative of pharmaceutical interest. These methods and this pharmaceutical compound can be applied/used in the pharmaceutical industry, hence they are to be considered industrially applicable according to article 33(4) PCT.

4. CLARITY (Art. 6 PCT).

4.1 The International Preliminary Examination Authority is of the opinion that the vague statement in the description on page 2 (see lines 21-23) implies that the subject-matter for which protection is sought may be different to that defined by the claims, thereby resulting in lack of clarity when used to interpret them (see also the PCT Guidelines, III-4.3a). In particular, the wording "*Further objects of the inventions will become obvious from... the appended claims*" relates to additional subject-matter, which is not clearly defined and does not fall within the scope of the claim.

REPLACED BY ART 34 AMDT

10 Rec'd PCT/PTO 28 JUN 2004

PEPTIDE PURIFICATION

FIELD OF THE INVENTION

The present invention relates to the purification of peptides, in particular intermediate-size peptides, more particularly nona- and decapeptides, such as LHRH-antagonists.

BACKGROUND OF THE INVENTION

Most intermediate size natural and synthetic peptides are amorphous substances. Many of them have pharmacologically interesting properties, such as many nona- and decapeptides which are LHRH (luteinizing hormone-releasing hormone) antagonists. One particular substance of this kind known only in amorphous form is the synthetic decapeptide of the formula (I)

Ac-D-2Nal-D-4ClPhe-D-3Pal-Ser-MeTyr-D-Asn-Leu-Lys(iPr)-Pro-D-Ala-NH_2 (I)

which, being a potent LHRH antagonist, has desirable pharmacological properties.

For use in pharmaceutical preparations it is necessary for the LHRH antagonist (I) and nona- and decapeptides of similar structure to be essentially pure. The raw product obtained in the last step of a multiple-step synthesis is purified by chromatographic and other methods. To eliminate residual solvent from the chromatography a thus purified product usually has to be dissolved in an aqueous medium and freeze-

dried. This is a costly process producing a voluminous product which is not easy to handle.

A process of purification of an otherwise pure peptide from residual organic solvent by other means than freeze-drying thus is desirable.

OBJECTS OF THE INVENTION

It is an object of the present invention to provide a process of purification of an otherwise pure peptide of the aforementioned kind from residual organic solvent which avoids freeze-drying.

It is another object of the present invention to provide such an otherwise pure peptide which is essentially free from residual organic solvent and is not in the form of a cryoprecipitate.

Further objects of the invention will become obvious from the following summary of the invention, the description of a preferred embodiment thereof, and the appended claims.

SUMMARY OF THE INVENTION

According to the present invention is provided a process of purification of an otherwise pure peptide, in particular a nona- or decapeptide, most particularly a nona- or decapeptide which is an LHRH antagonist, from residual organic solvent, comprising the following steps:

- dissolving said otherwise pure peptide in a dissolution solvent mixture comprising water and at least one alcohol selected from methanol, ethanol, propanol, isopropanol;
- adding the solution of the otherwise pure peptide in said solvent mixture to a vigorously stirred precipitation solvent mixture essentially consisting of one or several polar compounds selected from methyl acetate, ethyl acetate, methyl propionate, ethyl propionate, ethyl isopropionate, butyl acetate, isobutyl acetate, t-butyl acetate, ethyl formate, propyl formate, isopropyl formate, and one or several non-polar compounds selected from hexane, heptane, octane, cyclohexane, methylcyclohexane, and, optionally, of up to 5% of acetic or propionic acid;
- isolating the precipitated peptide;
- washing the isolated peptide with one or a mixture of said polar compounds or a solvent or solvent mixture of similar polarity,
- drying the washed peptide,

with the provisio that the water content of said solvent mixture comprising water and at least one alcohol is below 8% (v/v), and that the volume ratio of the dissolution solvent mixture and the precipitation solvent mixture is 1:10 or more.

"An otherwise pure peptide" is a peptide which is sufficiently pure for use in a medicine except for volatile impurities which need to be removed or the content of which needs to be substantially reduced. The otherwise pure peptide will normally be a substance having undergone purification by chromatography.

Preferably the otherwise pure peptide is

Ac-D-2Nal-D-4ClPhe-D-3Pal-Ser-MeTyr-D-Asn-Leu-Lys(iPr)-Pro-D-Ala-NH_2 (I).

According to a first preferred aspect of the invention the water content of the dissolution solvent mixture is below 5% (v/v).

According to a second preferred aspect of the invention the volume ratio of the dissolution solvent mixture and the precipitation solvent mixture is at least 15, in particular at least 20.

According to a third preferred aspect of the invention the alcohol of the dissolution solvent mixture is ethanol.

According to a fourth preferred aspect of the invention the polar component of the precipitation solvent mixture is ethyl acetate.

According to a fifth preferred aspect of the invention the non-polar component of the precipitation solvent mixture is heptane.

In the following the invention will be described in greater detail by reference to a preferred embodiment thereof which should not be understood to limit the invention.

DESCRIPTION OF A PREFERRED EMBODIMENT

Example 1. The fractions containing pure compound (1) (100 g in total) in ethanol-water-acetic acid 40:59:1 (v/v/v) obtained from preparative HPLC by which the synthetically obtained product had been purified were pooled and concentrated *in vacuo* to an oil which was co-evaporated twice from ethanol. The resulting solid was dissolved in 440 ml of ethanol and the resulting clear solution added over a period

of 20 min to 8.8 L of ethyl acetate/heptane 1:1 (v/v). Stirring was continued for 1 hour and filtered. The amorphous product was washed with 3 L of ethyl acetate; it was found that this removed nearly all heptane. The washed product was dried in a vacuum oven at 40oC at 0.3 bar for 48 hrs. Elemental analysis of the dried product indicated that the monoacetate of (I) had be obtained. Cryoprecipitation, in contrast, produces the corresponding diacetate. In the following Table analytical parameters of the monoacetate of (I) produced according to the invention are compared with those of a corresponding lyophilized product (diacetate).

Table. All percentages are by weight

	lyophilized product	precipitated product
Water	3.4 %	3.0%
Ethanol	<0.024%	<0.024%
Ethyl acetate	<0.024%	<0.024%
Heptane	<0.024%	<0.024%
Acetic acid (as acetate)	7.5%	3.9%
HPLC purity	99.8%	99.8%
Peptide content	88.8%	94.9%
Loss in filtrate	-	0.3%

Example 2. Variation of the composition of the precipitation solvent or solvent mixture; otherwise, procedure as in Example 1. Precipitation in pure ethyl acetate results in from about 3% to about 5% by weight loss of peptide. Precipitation in pure heptane results in a sticky product which is difficult to filter. A 1:1 (v/v) mixture of ethyl-acetate hexane gives a product which is easy to filter and dry; in repeated experiments the loss of peptide was always less than 0.5% by weight.

Example 3.Variation of water content of the solution of oily product in the dissolution solvent (absolute ethanol); otherwise, procedure as in Example 1. A water content of 10% (v/v) results in a sticky product on precipitation which is difficult to filter. A water content of 15% (v/v) results in an oily product on precipitation. To obtain satisfactory results the water content must not exceed 8% (v/v) but should preferably be kept below 5% (v/v). A water content below 5% is accomplished by co-evaporating the oily product from the chromatography at least twice with ethanol.

Example 4. Variation in precipitation temperature; otherwise, procedure as in Example 1. The precipitation temperature proved to be not critical. It could be varied from 0°C to 20°C without noticeable differences in product yield and morphology.

Example 5. Variation of concentration of (I) in the dissolution solvent; otherwise, procedure as in Example 1. It was found that the concentration of the oily product from the chromatography which had been co-evaporated with ethanol in the dissolution solvent should be as high as possible. Even a concentration of 330 g by weight could be used.

Example 6. Variation of ratio between dissolution solvent and precipitation solvent volumes and other variations; otherwise, procedure as in Example 1. The optimum ratio was found to be about 1:20. It could be shown that ratios from 1:15 to 1:30 gave satisfactory results. A ratio of 1:10 resulted in a sticky product. Precipitation is very fast. The suspension can be filtered 30 min after the last addition of dissolved substance. Washing with ethyl acetate did not result in loss of product but efficiently removed heptane.

CLAIMS

1. A process of purification of an otherwise pure nona- or decapeptide from residual organic solvent, comprising the following steps:
- dissolving the nona- or decapeptide in a dissolution solvent mixture comprising water and at least one alcohol selected from methanol, ethanol, propanol, isopropanol;
- adding the solution of the nona- or decapeptide in said solvent mixture to a vigorously stirred precipitation solvent mixture essentially consisting of one or several polar compounds selected from methyl acetate, ethyl acetate, methyl propionate, ethyl propionate, ethyl isopropionate, butyl acetate, isobutyl acetate, t-butyl acetate, ethyl formate, propyl formate, isopropyl formate, and one or several non-polar compounds selected from hexane, heptane, octane, cyclohexane, methylcyclohexane, and, optionally, of up to 5% of acetic or propionic acid;
- isolating the precipitated nona- or decapeptide;
- washing the isolated nona- or decapeptide with one or a mixture of said polar compounds or a solvent or solvent mixture of similar polarity,
- drying the washed nona- or decapeptide,
with the provisio that the water content of said solvent mixture comprising water and at least one alcohol is below 8% (v/v), and that the volume ratio of the dissolution solvent mixture and the precipitation solvent mixture is 1:10 or more.

2. The process of claim 1, wherein the water content of said solvent mixture comprising water and at least one alcohol is below 5% (v/v).

3. The process of claim 1, wherein said nona- or decapeptide is an LHRH antagonist.

4. The process of claim 3, wherein said nona- or decapeptide is Ac-D-2Nal-D-4ClPhe-D-3Pal-Ser-MeTyr-D-Asn-Leu-Lys(iPr)-Pro-D-Ala-NH_2 (I).

5. The process of claim 4, wherein Ac-D-2Nal-D-4ClPhe-D-3Pal-Ser-MeTyr-D-Asn-Leu-Lys(iPr)-Pro-D-Ala-NH_2 (I) is obtained in form of the monoacetate.

6. The process of claim 1, wherein the water content of the dissolution solvent mixture is below 5% (v/v).

7. The process of claim 1, wherein the volume ratio of the dissolution solvent mixture and the precipitation solvent mixture is at least 15.

8. The process of claim 1, wherein the alcohol of the dissolution solvent mixture is ethanol.

9. The process of claim 1, wherein the polar component of the precipitation solvent mixture is ethyl acetate.

10. The process of claim 1, wherein the non-polar component of the precipitation solvent mixture is heptane.

11. The monoacetate of Ac-D-2Nal-D-4ClPhe-D-3Pal-Ser-MeTyr-D-Asn-Leu-Lys(iPr)-Pro-D-Ala-NH_2.